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ORIGINAL COMMUNICATION

Effects of moderate alcohol consumption on folate and vitamin B_{12} status in postmenopausal women

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Background: Although alcohol intake has been positively associated with breast cancer risk in epidemiologic studies, a causal relationship has not been established, and the mechanisms mediating this association are speculative. Alcohol may act through altered status of folate and vitamin B_{12}, two vitamins required for DNA methylation and nucleotide synthesis, and thus cell integrity. Although the effects of heavy alcohol intake on folate and vitamin B_{12} status have been well-documented, few studies have addressed the effects of moderate alcohol intake in a controlled setting.
Objective: The objective of this study was to determine the effects of moderate alcohol intake on folate and vitamin B_{12} status in healthy, well-nourished, postmenopausal women.
Design: The study design was a randomized, diet-controlled crossover intervention. Postmenopausal women ($n=53$) received three 8-week alcohol treatments in random order: 0, 15, and 30 g/day. Treatment periods were preceded by 2–5-week washout periods. Blood collected at baseline and week 8 of each treatment period was analyzed for serum folate, vitamin B_{12}, homocysteine (HCY), and methylmalonic acid (MMA) concentrations.
Results: After adjusting for body mass index (BMI), a significant 5% decrease was observed in mean serum vitamin B_{12} concentrations from 0 to 30 g of alcohol/day (461.45 ± 30.26 *vs* 440.25 ± 30.24 pg/ml; $P=0.03$). Mean serum HCY concentrations tended to increase by 3% from 0 to 30 g of alcohol/day (9.44 ± 0.37 *vs* 9.73 ± 0.37 μmol/l; $P=0.05$). Alcohol intake had no significant effects on serum folate or MMA concentrations.
Conclusions: Among healthy, well-nourished, postmenopausal women, moderate alcohol intake may diminish vitamin B_{12} status.
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Introduction

Recent studies have shown a 40–100% increased risk of breast cancer for moderate alcohol drinkers (approximately 2–3 drinks per day) compared to nondrinkers (Sneyd *et al*, 1991; Zaridze *et al*, 1991; Friedenreich *et al*, 1993; Rosenberg *et al*, 1993; Katsouyanni *et al*, 1994; Longnecker, 1994; Longnecker *et al*, 1995; Smith-Warner *et al*, 1998; Zhang *et al*, 1999), and have indicated dose–response effects that do not appear to vary by type of alcohol (Longnecker, 1994; Smith-Warner *et al*, 1998). It has been suggested that alcohol may act through altering the status of folate and/or vitamin B_{12} due to their critical roles in one-carbon metabolism (Bailey & Gregory, 1999; Choi & Mason, 2000). Low folate intake and high alcohol intake have been shown to increase risk for colorectal cancer in several studies (Longnecker *et al*, 1990; Kune & Vitetta, 1992; Su & Arab, 2001; Giovannucci 2002;

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La Vecchia *et al*, 2002). In this cycle, the folate and vitamin B_{12}-dependent enzyme methionine synthase stands at the junction of two key processes responsible for maintaining DNA integrity: synthesis of purines and thymine, and methylation reactions via *S*-adenosylmethionine. A depletion of folate and/or vitamin B_{12} will therefore result in alterations in one-carbon metabolism and possibly procarcinogenic effects, including uracil misincorporation and increased susceptibility of DNA to strand breaks, aberrations in the methylation of DNA, and disruption of DNA repair (Bailey & Gregory, 1999; Choi & Mason, 2000).

Epidemiologic studies of folate and vitamin B_{12} status and breast cancer have indicated a negative association between folate *intake* and risk of breast cancer (Graham *et al*, 1991; Freudenheim *et al*, 1996; Ronco *et al*, 1999; Zhang *et al*, 1999; Rohan *et al*, 2000), as well as a negative association between vitamin B_{12} *status* and risk of breast cancer (Wu *et al*, 1999) and DNA damage (Fenech *et al*, 1998). The inverse association between folate status and breast cancer risk has been particularly strong among women who consumed alcohol (Sellers *et al*, 2001; Zhang *et al*, 2003). To determine whether alcohol intake is also inversely associated with the status of these vitamins, potentially bridging the gap between alcohol and breast cancer, many researchers have investigated the effects of *chronic* alcohol consumption on folate and vitamin B_{12} status (Eichner & Hillman, 1973; Halsted *et al*, 1973; Paine *et al*, 1973; McGuffin *et al*, 1975; Wu *et al*, 1975; Bonjour, 1980; Halsted, 1980; Lindenbaum, 1980). However, these studies are limited by small sample sizes, weaknesses associated with case–control and cross-sectional designs, failure to adjust for potentially confounding nutrient intakes, and the use of unreliable measures of folate, vitamin B_{12}, and alcohol intakes, all of which have high day-to-day variability (Guthrie & Crocetti, 1985; Posner *et al*, 1992; Firth *et al*, 1998). In addition, the results cannot be considered an adequate means of describing the relationship between *moderate* alcohol consumption and folate and vitamin B_{12} status, because even when alcoholic subjects appear to be free of complicating disease, it is difficult to distinguish specific effects of alcohol from generalized liver and other organ damage.

The few studies that have focused on moderate alcohol consumption share some of the same limitations (Jacques *et al*, 1989; Tucker *et al*, 1992). Only one study (van der Gaag *et al*, 2000) used an experimental design and controlled diet to investigate the effects of moderate alcohol consumption; however, it was limited by an abbreviated period of alcohol administration, a small sample size, a lack of female representation, and a failure to assess dose-response. Our study was therefore designed to address the gaps in the literature using a randomized, diet-controlled, crossover intervention to determine what effects moderate alcohol consumption has on folate and vitamin B_{12} status in postmenopausal women. To corroborate the results of the more common serum folate and vitamin B_{12} assays, our study also measured serum homocysteine (HCY) and serum methylmalonic acid (MMA), two metabolites that have been found to be sensitive functional indicators of folate and vitamin B_{12} status (Savage *et al*, 1994; Stabler *et al*, 1996; Klee, 2000).

Subjects and methods

Subjects

Subjects were recruited through advertisements placed in communities around Beltsville, MD. Participation began in 1998 and continued through 1999. To be eligible, subjects were required to meet the following criteria: (1) aged 49 y or older; (2) postmenopausal (last menses at least 1 y prior to the study and/or a follicle stimulating hormone concentration of 40 IU/ml or greater); (3) having at least one intact ovary; (4) not taking hormone replacement therapy; (5) not smoking; (6) having no major health problems, including a history of heart disease, stroke, diabetes, hypertension, or cancer (other than nonmelanoma skin cancer); (7) having 90–140% of ideal weight-for-height; (8) having no history of alcohol use problems, but not an abstainer; (9) having no family history of alcoholism; (10) having no food allergies; (11) willing to avoid prescription medications that could interfere with the study; and (12) willing to eat all of the foods and only the foods supplied by the study. Subjects were required to discontinue the use of any multivitamin and other supplements, including folic acid and vitamin B_{12}, at least 4 weeks prior to the start of the study and throughout their participation in the research. Before entering the study, all subjects signed an informed consent form. The study was approved by the institutional review boards at the National Cancer Institute (Bethesda, MD, USA) and the Johns Hopkins University School of Public Health (Baltimore, MD, USA). A total of 65 women completed baseline visits. Of these, two women dropped out after the baseline visit, eight women dropped out during the first dietary period, and two women dropped out during the washout period that followed, leaving a total of 53 women for analysis.

Study design

Using a crossover design, all subjects received a controlled diet and three 8-week alcohol treatments in random order: 0 g/day (control), 15 g/day (approximately one drink), and 30 g/day (approximately two drinks). The 30 g/day level was selected based on biomarker response in a previous study (Reichman *et al*, 1993), the 15 g/day was selected based on current dietary recommendations (Dietary Guidelines Advisory Committee, 2000), and the 0 g/day was selected as a control. These three levels provided an opportunity to evaluate dose-response. Each dietary period was preceded by a 2–5-week washout period during which the women consumed no alcohol without other dietary restrictions, other than they were asked not to consume any dietary supplements.

All food and beverages, including alcoholic beverages, were prepared and supplied by the U.S. Department of Agriculture's Beltsville Human Nutrition Research Center

Human Study Facility. Alcohol was supplied as 95% ethyl alcohol (Everclear™; Pharmco Products, Inc., Brookfield, CT, USA) in orange juice (12 ounces). The subjects were instructed to consume the alcoholic beverages with a snack (provided by the study) approximately 1–2 h before bedtime after completing any activities that required manual dexterity. Subjects were blinded as to the specific alcohol content of their beverages. During the 0- and 15-g alcohol treatment periods, energy from alcohol was replaced with energy from carbohydrates (Polycose™; Abbott Laboratories, Columbus, OH, USA). Twice during each of the three treatment periods, compliance was measured at random by adding 40 mg of riboflavin to the beverages followed by collection of unannounced morning urine samples for a qualitative determination for riboflavin. The following morning, subjects provided a urine sample that was checked for the presence of riboflavin by fluorescence under black light.

The controlled diet consisted of approximately 15% energy as protein, 50% energy as carbohydrates, and 35% energy as fat, with a polyunsaturated to monounsaturated to saturated fat ratio of 0.6:1:1. The diets also provided 100% of the Dietary Reference Intakes (DRI) for all vitamins and minerals. With the exception of calcium and iron, supplements were prohibited. Daily dietary fiber and cholesterol intakes were 10 g and 150 mg per 1000 kcal, respectively. During the study, weight was measured each weekday and kcal intake was adjusted in 200-kcal increments as needed to maintain body weight. On weekdays, morning and evening meals were consumed in the Center's dining facility and a carryout lunch was provided. Weekend meals were packed for home consumption. Meals were prepared from typical foods using a 7-day menu cycle.

Laboratory methods

Blood for folate, vitamin B_{12}, HCY, and MMA analyses was collected between 0630 and 0900 hours after an overnight fast at baseline and once during the last week of each treatment period. Serum was separated, and aliquots were frozen at -70°C. Samples for individual subjects were grouped in random order and were analyzed together in the same batch. For quality control, 11 aliquots from a pooled serum sample were randomly inserted among study samples, but not within any individual subject's set of three samples. Coefficients of variation on masked quality control samples included in each batch were as follows: vitamin $B_{12} = 3.7\%$, folate $= 2.9\%$, HCY $= 2.9\%$, and MMA 11.0%.

All laboratory analyses were performed by the NHANES Laboratory at the Centers for Disease Control and Prevention (Atlanta, GA, USA). Concentrations of folate and vitamin B_{12} in serum were measured using BioRad Laboratories 'Quantaphase II' tandem ^{125}I-Folate/ ^{57}Co-vitamin B_{12} radioassay kits (BioRad Laboratories, Hercules, CA, USA) (Gunter *et al*, 1996), which measures total folate levels. Serum HCY was analyzed by HPLC with pre-column derivatization and fluorescence detection according to the method described by Pfeiffer *et al* (1999), using cystamine dihydrochloride (internal standard), trichloroacetic acid containing EDTA for deproteinization, and ammonium 7-fluorobenzo-2-oxa-1,3-diazole-4-sulfonate (SBD-F, Wako Chemicals, Richmond, VA, USA) added for derivatization. Calibration was performed daily and was evaluated as both external and internal calibration (area ratios between the thiol and the internal standard, cystamine dihydrochloride).

Serum MMA was analyzed by the gas chromatography-mass spectrometry method validated by Pfeiffer and Gunter (Pfeiffer & Gunter, 1999) and used for a subset of the National Health and Nutrition Examination Survey (NHANES 1999–2003). Results were quantified with an external two-point calibration curve using peak area ratios of MMA and the internal standard (d3-MMA).

Statistical analysis

Linear mixed model regression analysis (Laird & Ware, 1982) was used to estimate differences in folate, vitamin B_{12}, HCY, and MMA concentrations between alcohol treatments (0, 15, 30 g). The subject was treated as a random effect (a single random intercept) and alcohol levels were treated as fixed effects designated by two indicator variables. Nutrient concentrations were analyzed as untransformed and transformed to the $\log_e$. Since the outcomes were similar, the untransformed results are reported. To assess the effect of model assumptions on study results, Student's paired *t*-test was also used to evaluate changes in nutrient concentrations by alcohol treatment.

Likelihood ratio tests were used to test for trend and also to evaluate whether covariates improved model fit. A model with a three-level treatment variable (0 drinks/1 drink/2 drinks) was compared to a model with a two-level treatment variable (yes/no alcohol) to test for trend. Potential covariates, including age, race, BMI (weight in kg/[height in m]^2), treatment order, sample batch, dietary period, dietary intake of nutrients at baseline, and years since menopause, were evaluated for contribution to overall fit or improvement in the precision of the model. Standard errors of alcohol parameter estimates from simple models were compared to those from models that included characteristics statistically significantly associated with each nutrient, in order to evaluate the effect of adjustment on precision. BMI improved precision and was included in the final model. The other covariates evaluated had no effect on the model.

Effect modification by treatment order, dietary period, BMI, baseline nutrient status, and age was assessed by likelihood ratio tests of improvement in model fit after addition of the cross product terms to models that included the main effects for alcohol and the characteristics of interest. None of these variables had an effect on model fit or precision. A two-tailed P of <0.05 was used to define a significant association. All analyses were performed using SAS (SAS/STAT version 6, SAS Institute, Inc., 1996).

Results

Baseline characteristics of the subjects are summarized in Table 1. Both serum folate and vitamin B_{12} were inversely related to serum HCY ($R=-0.55$; $P<0.001$ and $R=-0.68$; $P<0.001$, respectively). Vitamin B_{12} was also inversely related to serum MMA ($R=-0.77$; $P<0.001$).

The subjects' prestudy mean daily intakes of energy from protein (15%), fat (30%), and carbohydrates (57%) were fairly typical and were similar to those of the controlled diet during the 0-g alcohol treatment (data not presented). A total of 10 women reported using multivitamins prior to entering into the study, and no women consumed individual supplements of the nutrients of interest (data not presented). As directed, women discontinued these supplements at least 4 weeks prior to beginning the study; therefore, at entry, none of the subjects reported consuming supplements containing folic acid or vitamin B_{12}. Compared to the current DRI supplied by the controlled diet (Food and Nutrition Board, 2000), the subjects' estimates of their usual diets showed that, on average, intakes of several nutrients, including folate but not vitamin B_{12}, fell below the recommendations. When Student's paired *t*-tests were used to estimate changes in serum concentrations of folate, vitamin B_{12}, HCY, and MMA from the prestudy baseline to the 0 g/day alcohol treatment, the results showed that serum HCY concentrations significantly decreased (10.03 *vs* 9.45 μmol/l; $P=0.02$) while serum folate concentrations tended to increase (15.77 *vs* 17.37 ng/ml; $P=0.09$). The qualitative test for alcohol intake suggested that all subjects had excellent compliance.

Table 2 shows the mean differences in folate, vitamin B_{12}, HCY, and MMA concentrations from 0 to 15, 0 to 30, and 15 to 30 g alcohol/day for all subjects after adjusting for BMI. Consumption of 15 g alcohol/day compared to 0 g alcohol/day was associated with a significant 5% decrease in serum vitamin B_{12} concentrations ($P=0.04$). However, no significant changes in serum concentrations of folate, HCY, or MMA were observed between these treatments. Consumption of 30 g alcohol/day compared to 0 g alcohol/day was also associated with a significant 5% decrease in serum vitamin B_{12} concentrations ($P=0.03$). In addition, serum HCY concentrations tended to increase by 3% from 0 to 30 g alcohol/day ($P=0.05$). No significant differences in serum concentrations of folate or MMA were observed between these two treatments. None of variables examined showed significant differences for the 15 *vs* 30 g alcohol/day comparisons. Comparison of the two test-for-trend models (0 drinks/1 drink/2 drinks; yes/no alcohol) revealed no significant differences.

Table 1 Subject characteristics at baseline ($n=53$)

Characteristic	*Mean*	*Range*
Age (y)	59.7	49–79
Height (cm)	163.7	152.1–179.7
Weight (kg)	74.4	42.1–117.4
Body mass index (kg/m^2)	27.8	17.7–42.5
Alcohol intake before study (# drinks/week)	0.9	0–15
Serum folate (ng/ml)	15.8	4.0–38.5
Serum vitamin B_{12} (pg/ml)[a]	470.7	105.0–2694.0
Serum homocysteine (μmol/l)	10.0	5.7–20.0
Serum methylmalonic acid (μmol/l)	0.24	0.088–1.46

Characteristic	*No.*	*%*
Race		
Caucasian	39	73.6
African American	12	22.6
Asian	2	3.8

[a]The mean value for serum vitamin B_{12} is skewed to the right because it includes the outlier 2694.0 pg/ml, which is much higher than the second highest value, 979.0 pg/ml. All analyses were performed with and without this outlier. Since the results were not different, it was included. The serum values above are for the analytes at entry into the study.

Discussion

This is the first study to use a randomized, diet-controlled, crossover trial to determine the effects of moderate alcohol consumption on folate and vitamin B_{12} status in postmenopausal women. Our findings indicate that moderate alcohol consumption may have adverse effects on vitamin B_{12} status. Compared to consumption of 0 g of alcohol/day, consumption of 15 or 30 g of alcohol/day for 8 weeks was associated with a statistically significant 5% decrease in serum vitamin

Table 2 Least-squares mean serum nutrient concentrations by alcohol treatment and least-squares mean changes between alcohol treatments—maximum likelihood mixed model regression analysis[a,b] ($n=53$)

	Least-squares mean (g/day)			*Change in least-squares mean (g/day)*		
Nutrient	0	15	30	0–15	0–30	15–30
Folate (ng/ml)	17.37 ± 0.77	17.32 ± 0.77	17.97 ± 0.77	−0.043 ± 0.64	0.60 ± 0.63	0.65 ± 0.63
Vitamin B_{12} (pg/ml)	461.45 ± 30.26	441.31 ± 30.26	440.25 ± 30.24	−20.13* ± 9.86	−21.20† ± 9.79	−1.07 ± 9.78
Homocysteine (μmol/l)	9.44 ± 0.37	9.54 ± 0.37	9.73 ± 0.37	0.098 ± 0.15	0.29‡ ± 0.15	0.19 ± 0.15
Methylmalonic acid (μmol/l)	0.22 ± 0.02	0.21 ± 0.02	0.22 ± 0.02	−0.0077 ± 0.009	0.0072 ± 0.009	0.015 ± 0.009

[a]Mean ± s.e.m.
[b]Model adjusted for BMI.
*$P=0.04$; †$P=0.03$; ‡$P=0.05$.

B_{12} concentrations. Consumption of 30 g of alcohol/day for 8 weeks also tended to increase serum HCY concentrations by 3%. Moderate alcohol consumption did not significantly affect serum folate or MMA concentrations at any dose. There was no indication of dose-response for any of the variables tested.

There has only been one other reported study with a similar randomized diet-controlled crossover design (van der Gaag *et al*, 2000). In that study, 11 men consumed 40 g/day of spirits, wine, beer, or water for 3 weeks each, in addition to a nutritionally adequate diet. Although consumption of spirits was associated with a decrease in serum vitamin B_{12}, the change was not significant. In contrast, consumption of spirits was associated with a significant 10% decrease in serum folate, and spirits and wine were each associated with a significant 8% increase in serum HCY. The changes in serum HCY compare favorably with the changes in our study. It is likely that the changes in serum vitamin B_{12} did not reach statistical significance as a result of design limitations, including mainly the small sample size but also the abbreviated period of alcohol administration. It is also possible that the folate and vitamin B_{12}-sufficient diet countered some of the effects of alcohol on serum vitamin B_{12}.

Indeed, the results of all of the most recent studies dealing with alcohol's effects on folate and vitamin B_{12} status seem to suggest that alcohol consumption and inadequate dietary intake may act synergistically to deplete serum folate and vitamin B_{12} concentrations. Otherwise, a nutritionally adequate diet may oppose any effects of alcohol. A case–control study and a cross-sectional analysis both showed significantly lower folate concentrations in alcoholics (Cravo *et al*, 1996; Gloria *et al*, 1997). Neither study adjusted for nutrient intakes, which were below the DRI in 56% of the alcoholics in the cross-sectional analysis (Gloria *et al*, 1997) and most likely lower than that of the healthy controls in the case–control study (Cravo *et al*, 1996). Other studies of alcohol's effects on folate metabolism in humans and rats support this theory. In one study, jejunal uptake of labeled folate was significantly lower in alcoholics consuming ethanol in conjunction with a folate-deficient diet compared to alcoholics consuming either ethanol with a folate-sufficient diet or a folate-deficient diet without ethanol (Halsted *et al*, 1971, 1973). Similarly, rats fed folate-deficient diets supplemented with ethanol had significantly lower concentrations of biliary folates compared to ethanol-treated but folate-replete rats (Hillman *et al*, 1977; Weir *et al*, 1985). Some recent research has suggested that polymorphic genes involved in folate metabolism (eg methylene tetrahydrofolate reductase—MTHFR) may modify the relationship between dietary folate and breast cancer risk (Sharp *et al*., 2002; Semenza *et al*, 2003) as had previously been noted for colorectal cancer (Giovannucci, 2002). We have not evaluated the possibility that these polymorphisms may have modified folate metabolism overall or modified the role of alcohol on folate metabolism in our study due to the relatively small number of subjects.

In contrast to studies of alcohol intake and poor diet, studies that involved adequate nutrient intakes showed nonsignificant changes in serum concentrations of folate and vitamin B_{12}. A cross-sectional analysis of 586 well-nourished elderly showed that those subjects with a daily alcohol intake of 15 g or more exhibited lower serum vitamin B_{12} concentrations compared to those consuming 0–4 g, but the difference was not statistically significant (Jacques *et al*, 1989). Serum folate concentrations were not affected by alcohol intake in this population. Similarly, a cluster analysis of 680 well-nourished elderly showed that the alcohol cluster (19.5% of kcal from alcohol) had comparable or even higher nutrient intakes than the other three clusters: milk/cereal/fruit, bread/poultry, and meat/potatoes (1.5% of kcal from alcohol) (Tucker *et al*, 1992). The results indicated that the alcohol cluster exhibited lower plasma vitamin B_{12} concentrations than all of the other clusters, but the difference only reached statistical significance compared to the milk/cereal/fruit cluster. The alcohol cluster even had significantly higher plasma folate concentrations compared to the bread/poultry and meat/potatoes clusters. Finally, a controlled, nutritionally adequate diet study showed that 10 rats that received alcohol as 36% of kcal for 4 weeks exhibited lower plasma folate and vitamin B_{12} concentrations compared to 10 rats that received an equivalent amount of maltodextrin, but the differences between the two groups did not reach statistical significance (Stickel *et al*, 2000). The alcohol-fed rats did exhibit significantly higher concentrations of serum HCY (37%), which again compares favorably with our findings.

Taken together, the results of the above studies suggest that inadequate vitamin intakes and alcohol consumption may act to deplete folate and vitamin B_{12} status. In our study, the controlled diet supplied 100% or more of the DRI for all vitamins and minerals. Thus, the negative effects of alcohol observed in previous studies of alcohol intake and poor diet may have been masked in our study due to the folate-replete diet. Wu *et al* (1999) reported median values for serum folate, vitamin B_{12}, and homocysteine levels among 85 control participants in a nested case–control study of breast cancer among Maryland women. Their results for their 1989 cohort group with median values of 8.0 ng/ml for folate, 452 pg/ml for B_{12}, and 9.2 nmol/ml for HCY, respectively, suggest that even at baseline, our subject population consumed diets that were particularly replete in folic acid.

Vitamin B_{12} intakes were similar before and during the study, and, unlike serum folate concentrations, serum vitamin B_{12} concentrations did not change with the introduction of the controlled diet. Therefore, the effects of alcohol on serum vitamin B_{12} concentrations in our study may have been more apparent. Despite the nutritionally adequate diet, our findings indicated that vitamin B_{12} status may be adversely affected by alcohol, and the observed changes can most likely be attributed to improvements in design over previous studies. One improvement was the additional measurement of serum HCY, the increase of

which supports the observed change in serum vitamin B_{12}. The association between alcohol intake and vitamin B_{12} status has been attributed to intestinal malabsorption, and, more specifically, to altered binding of intrinsic factor (Shaw *et al*, 1990) and alcohol-induced ileal damage (Lindenbaum, 1980).

It is unknown whether the 5% decrease in serum vitamin B_{12} and the 3% increase in serum HCY represent biologically meaningful changes. It is also uncertain whether the lack of change in serum folate would persist in the absence of a controlled diet. These observations were made over a relatively short period of time among healthy women consuming a nutritionally adequate diet. Presumably, however, many years of moderate alcohol intake in combination with a poor diet could produce more substantial changes. Outside the controlled environment of our study, inadequate nutrient intakes are fairly common, especially with respect to folate (Ford & Ballew, 1998; Ford & Bowman, 1999; Kant & Schatzkin, 1999). Therefore, our findings may be underestimated with respect to the general population.

Potential limitations of our study include the moderately brief treatment periods and a small number of alcohol doses. Future research might include longer treatment periods, different types of alcoholic beverages, and possibly more doses of alcohol. In summary, we observed that among healthy, well-nourished, postmenopausal women, moderate alcohol intake may have adverse effects on vitamin B_{12} status.

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References

Bailey LB & Gregory III JF (1999): Folate metabolism and requirements. *J. Nutr.* **129**, 779–782.

Bonjour JP (1980): Vitamins and alcoholism. II. folate and vitamin B12. *Int. J. Vitam. Nutr. Res.* **50**, 96–121.

Choi SW & Mason JB (2000): Folate and carcinogenesis: an integrated scheme. *J. Nutr.* **130**, 129–132.

Cravo ML, Gloria LM, Selhub J, Nadeau MR, Camilo ME, Resende MP, Cardoso JN, Leitao CN & Mira FC (1996): Hyperhomocysteinemia in chronic alcoholism: correlation with folate, vitamin B-12, and vitamin B-6 status. *Am. J. Clin. Nutr.* **63**, 220–224.

Dietary Guidelines Advisory Committee (2000): Report of the Dietary Guidelines advisory committee on the Dietary Guidelines for Americans, 2000. Washington, DC: US Department of Health and Human Services.

Eichner ER & Hillman RS (1973): Effect of alcohol on serum folate level. *J. Clin. Invest.* **52**, 584–591.

Fenech M, Aitken C & Rinaldi J (1998): Folate, vitamin B_{12}, homocysteine status and DNA damage in young Australian adults. *Carcinogenesis* **19**, 1163–1171.

Firth Y, Murtaugh MA & Tangney CC (1998): Estimation of individual intakes of folate in women of childbearing age with and without simulation of folic acid fortification. *J. Am. Diet. Assoc.* **98**, 985–988.

Food and Nutrition Board (2000) Dietary Reference Intakes for Thiamin, Riboflavin, Niacin, Vitamin B6, Folate, Vitamin B12, Pantothenic Acid, Biotin, and Choline. A Report of the Standing Committee on the Scientific Evaluation of Dietary Reference Intakes and its Panel on Folate, Other B Vitamins, and Choline, and Subcommittee on Upper Reference Levels for Nutrients, Food and Nutrition Board, Institute of Medicine. Washington, DC: National Academy Press.

Ford ES & Ballew C (1998): Dietary folate intake in US adults: findings from the third National Health and Nutrition Examination Survey. *Ethn. Dis.* **8**, 299–305.

Ford ES & Bowman BA (1999): Serum and red blood cell folate concentrations, race, and education: findings from the third National Health and Nutrition Examination Survey. *Am. J. Clin. Nutr.* **69**, 476–481.

Freudenheim JL, Marshall JR, Vena JE, Laughlin R, Brasure JR, Swanson MK, Nemoto T & Graham S (1996): Premenopausal breast cancer risk and intake of vegetables, fruits, and related nutrients. *J. Natl. Cancer Inst.* **88**, 340–348.

Friedenreich CM, Howe GR, Miller AB & Jain MG (1993): A cohort study of alcohol consumption and risk of breast cancer. *Am. J. Epidemiol.* **137**, 512–520.

Giovannucci E (2002): Epidemiologic studies of folate and colorectal neoplasia: a review. *J. Nutr.* **132**, 2350S–2355S.

Gloria L, Cravo M, Camilo ME, Resende M, Cardoso JN, Oliveira AG, Leitao CN & Mira FC (1997): Nutritional deficiencies in chronic alcoholics: relation to dietary intake and alcohol consumption. *Am. J. Gastroenterol.* **92**, 485–489.

Graham S, Hellmann R, Marshall J, Freudenheim J, Vena J, Swanson M, Zielezny M, Nemoto T, Stubbe N & Raimondo T (1991): Nutritional epidemiology of postmenopausal breast cancer in western New York. *Am. J. Epidemiol.* **134**, 552–566.

Gunter EW, Lewis BL & Koncikowski SM (1996): *Laboratory methods used for the Third National Health and Nutrition Examination Survey (NHANES III), 1988–1994. Included in CD-ROM 6-0178, NHANES III Reference Manuals and Reports*. Hyattsville, MD: Centers for Disease Control and Prevention. pp VII-D-1–VII-D-14.

Guthrie HA & Crocetti AF (1985): Variability of nutrient intake over a 3-day period. *J. Am. Diet. Assoc.* **85**, 325–327.

Halsted CH (1980): Folate deficiency in alcoholism. *Am. J. Clin. Nutr.* **33**, 2736–2740.

Halsted CH, Robles EA & Mezey E (1971): Decreased jejunal uptake of labeled folic acid (3 H-PGA) in alcoholic patients: roles of alcohol and nutrition. *N. Engl. J. Med.* **285**, 701–706.

Halsted CH, Robles EA & Mezey E (1973): Intestinal malabsorption in folate-deficient alcoholics. *Gastroenterology* **64**, 526–532.

Hillman RS, McGuffin R & Campbell C (1977): Alcohol interference with the folate enterohepatic cycle. *Trans. Assoc. Am. Physicians* **90**, 145–156.

Jacques PF, Sulsky S, Hartz SC & Russell RM (1989): Moderate alcohol intake and nutritional status in nonalcoholic elderly subjects. *Am. J. Clin. Nutr.* **50**, 875–883.

Kant AK & Schatzkin A (1999): Relation of age and self-reported chronic medical condition status with dietary nutrient intake in the US population. *J. Am. Coll. Nutr.* **18**, 69–76.

Katsouyanni K, Trichopoulou A, Stuver S, Vassilaros S, Papadiamantis Y, Bournas N, Skarpou N, Mueller N & Trichopoulos D (1994): Ethanol and breast cancer: an association that may be both confounded and causal. *Int. J. Cancer* **58**, 356–361.

Klee GG (2000): Cobalamin and folate evaluation: measurement of methylmalonic acid and homocysteine *vs* vitamin B(12) and folate. *Clin. Chem.* **46**, 1277–1283.

Kune GA & Vitetta L (1992): Alcohol consumption and the etiology of colorectal cancer: a review of the scientific evidence from 1957 to 1991. *Nutr. Cancer.* **18**, 97–111.

Laird NM & Ware JH (1982): Random-effects models for longitudinal data. *Biometrics.* **38**, 963–974.

La Vecchia C, Negri E, Pelucchi C & Franceschi S (2002): Dietary folate and colorectal cancer. *Int. J. Cancer.* **102**, 545–547.
Lindenbaum J (1980): Folate and vitamin B12 deficiencies in alcoholism. *Semin. Hematol.* **17**, 119–129.
Longnecker MP (1994): Alcoholic beverage consumption in relation to risk of breast cancer: meta-analysis and review. *Cancer Causes Control* **5**, 73–82.
Longnecker MP, Orza MJ, Adams ME, Vioque J & Chalmers TC (1990): A meta-analysis of alcoholic beverage consumption in relation to risk of colorectal cancer. *Cancer Causes Control* **1**, 59–68.
Longnecker MP, Newcomb PA, Mittendorf R, Greenberg ER, Clapp RW, Bogdan GF, Baron J, MacMahon B & Willett WC (1995): Risk of breast cancer in relation to lifetime alcohol consumption. *J. Natl. Cancer Inst.* **87**, 923–929.
McGuffin R, Goff P & Hillman RS (1975): The effect of diet and alcohol on the development of folate deficiency in the rat. *Br. J. Haematol.* **31**, 185–192.
Paine CJ, Eichner ER & Dickson V (1973): Concordance of radioassay and microbiological assay in the study of the ethanol-induced fall in serum folate level. *Am. J. Med. Sci.* **266**, 134–138.
Pfeiffer CM & Gunter EW (1999): Automated assay for methylmalonic acid (MMA) in plasma. *Clin. Chem.* **45**, A166–A167.
Pfeiffer CM, Huff DL & Gunter EW (1999): Rapid and accurate HPLC assay for plasma total homocysteine and cysteine in a clinical laboratory setting. *Clin. Chem.* **45**, 290–292.
Posner BM, Martin-Munley SS, Smigelski C, Cupples LA, Cobb JL, Schaefer E, Miller DR & D'Agostino RB (1992): Comparison of techniques for estimating nutrient intake: the Framingham Study. *Epidemiology.* **3**, 171–177.
Reichman ME, Judd JT, Longcope C, Schatzkin A, Clevidence BA, Nair PP, Campbell WS & Taylor PR (1993): Effects of alcohol consumption on plasma and urinary hormone concentrations in premenopausal women. *J. Natl. Cancer Inst.* **85**, 722–727.
Rohan TE, Jain MG, Howe GR & Miller AB (2000): Dietary folate consumption and breast cancer risk. *J. Natl. Cancer Inst.* **92**, 266–269.
Ronco A, De Stefani E, Boffetta P, Deneo-Pellegrini H, Mendilaharsu M & Leborgne F (1999): Vegetables, fruits, and related nutrients and risk of breast cancer: a case-control study in Uruguay. *Nutr. Cancer* **35**, 111–119.
Rosenberg L, Metzger LS & Palmer JR (1993): Alcohol consumption and risk of breast cancer: a review of the epidemiologic evidence. *Epidemiol. Rev.* **15**, 133–144.
SAS Institute, Inc (1996): *SAS/STAT User's Guide* Version 6. Cary, NC: SAS Institute, Inc.
Savage DG, Lindenbaum J, Stabler SP & Allen RH (1994): Sensitivity of serum methylmalonic acid and total homocysteine determinations for diagnosing cobalamin and folate deficiencies. *Am. J. Med.* **96**, 239–246.
Sellers TA, Kushi LH, Cerhan JR, Vierkant RA, Gapstur SM, Vachon CM, Olson JE, Therneau TM & Folsom AR (2001): Dietary folate intake, alcohol, and risk of breast cancer in a prospective study of postmenopausal women. *Epidemiology* **12**, 420–428.
Semenza JC, Delfino RJ, Ziogas A & Anton-Culver H (2003): Breast cancer risk and methylenetatrahydrofolate reductase polymorphism. *Breast Cancer Res Treat* **77**, 217–223.
Sharp L, Little J, Schofield AC, Pavlidou E, Cotton SC, Miedzybrodzka Z, Baird JO, Haites NE, Heys SD & Grubb DA (2002): Folate and breast cancer: the role of polymorphisms in methylenetetrahydrofolate reductase (MTHFR). *Cancer Lett* **181**, 65–71.
Shaw S, Herbert V, Colman N & Jayatilleke E (1990): Effect of ethanol-generated free radicals on gastric intrinsic factor and glutathione. *Alcohol* **7**, 153–157.
Smith-Warner SA, Spiegelman D, Yaun SS, van den Brandt PA, Folsom AR, Goldbohm RA, Graham S, Holmberg L, Howe GR, Marshall JR, Miller AB, Potter JD, Speizer FE, Willett WC, Wolk A & Hunter DJ (1998): Alcohol and breast cancer in women: a pooled analysis of cohort studies. *JAMA* **279**, 535–540.
Sneyd MJ, Paul C, Spears GF & Skegg DC (1991): Alcohol consumption and risk of breast cancer. *Int. J. Cancer* **48**, 812–815.
Stabler SP, Lindenbaum J & Allen RH (1996): The use of homocysteine and other metabolites in the specific diagnosis of vitamin B-12 deficiency. *J. Nutr.* **126**, 1266S–1272S.
Stickel F, Choi SW, Kim YI, Bagley PJ, Seitz HK, Russell RM, Selhub J & Mason JB (2000): Effect of chronic alcohol consumption on total plasma homocysteine level in rats. *Alcohol Clin. Exp. Res.* **24**, 259–264.
Su LJ & Arab L (2001): Nutritional status of folate and colon cancer risk: evidence from NHANES 1 epidemiologic follow-up study. *Ann. Epidemiol.* **11**, 65–72.
Tucker KL, Dallal GE & Rush D (1992): Dietary patterns of elderly Boston-area residents defined by cluster analysis. *J. Am. Diet. Assoc.* **92**, 1487–1491.
van der Gaag MS, Ubbink JB, Sillanaukee P, Nikkari S & Hendriks HF (2000): Effect of consumption of red wine, spirits, and beer on serum homocysteine. *Lancet.* **355**, 1522.
Weir DG, McGing PG & Scott JM (1985): Folate metabolism, the enterohepatic circulation and alcohol. *Biochem. Pharmacol.* **34**, 1–7.
Wu A, Chanarin I, Slavin G & Levi AJ (1975): Folate deficiency in the alcoholic–its relationship to clinical and haematological abnormalities, liver disease and folate stores. *Br. J. Haematol.* **29**, 469–478.
Wu K, Helzlsouer KJ, Comstock GW, Hoffman SC, Nadeau MR & Selhub J (1999): A prospective study on folate, B12, and pyridoxal 5′-phosphate (B6) and breast cancer. *Cancer Epidemiol. Biomarkers Prev.* **8**, 209–217.
Zaridze D, Lifanova Y, Maximovitch D, Day NE & Duffy SW (1991): Diet, alcohol consumption and reproductive factors in a case–control study of breast cancer in Moscow. *Int. J. Cancer* **48**, 493–501.
Zhang S, Hunter DJ, Hankinson SE, Giovannucci EL, Rosner BA, Colditz GA, Speizer FE & Willett WC (1999): A prospective study of folate intake and the risk of breast cancer. *JAMA* **281**, 1632–1637.
Zhang SM, Willett WC, Selhub J, Hunter DJ, Giovannucci EL, Holmes MD, Colditz GA & Hankinson SE (2003): Plasma folate, vitamin B6, vitamin B12, homocysteine, and risk of breast cancer. *J. Natl. Cancer Inst.* **95**, 373–380.